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Genetic relationship between predisposition for binge alcohol consumption and blunted sensitivity to adverse effects of alcohol in mice

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Abstract

Background—Initial sensitivity to ethanol and the capacity to develop acute functional tolerance (AFT) to its adverse effects may influence the amount of alcohol consumed and may also predict future alcohol use patterns. The current study assessed sensitivity and AFT to the ataxic and hypnotic effects of ethanol in the first replicate of mice (HDID-1) selectively bred for high blood ethanol concentrations (BECs) following limited access to ethanol in the Drinking in the Dark (DID) paradigm.

Methods—Naïve male and female HDID-1 and HS/Npt mice from the progenitor stock were evaluated in three separate experiments. In experiments 1 and 2, ethanol-induced ataxia was assessed using the static dowel task. In experiment 3, ethanol-induced hypnosis was assessed by the method of Ponomarev and Crabbe (2002), using modified restraint tubes to measure the loss of righting reflex (LORR).

Results—HDID-1 mice exhibited reduced initial sensitivity to both ethanol-induced ataxia ($p < 0.001$) and hypnosis ($p < 0.05$) relative to HS/Npt mice. AFT was calculated by subtracting the BEC at loss of function from the BEC at recovery (Experiments 1 and 3) or by subtracting BEC at an initial recovery from the BEC at a second recovery following an additional alcohol dose (Experiment 2). The dowel test yielded no line differences in AFT, but HS/Npt mice developed slightly greater AFT to ethanol-induced LORR than HDID-1 ($p < 0.05$).

Conclusions—These results suggest that HDID-1 mice exhibit aspects of blunted ataxic and hypnotic sensitivity to ethanol which may influence their high ethanol intake via DID, but do not display widely different development of AFT. These findings differ from previous findings with the high alcohol-preferring (HAP) selected mouse lines, suggesting that genetic predisposition for binge, versus other forms of excessive alcohol consumption, is associated with unique responses to ethanol-induced motor incoordination.

INTRODUCTION

Alcohol use disorders (AUDs) have been shown to have a substantial genetic component (Kendler et al., 2011, Merikangas et al., 1998). Certain responses to alcohol that are influenced by genetic background (e.g. sensitivity and tolerance) may play an important role in an individual's alcohol use. It is possible that key differences among individuals in responses to alcohol that could aid in the assessment of abuse/dependence risk may be altered or no longer apparent following considerable alcohol exposure. It is therefore critical to evaluate responses to alcohol in pre-dependent individuals to determine how these responses may be associated with various types of future problematic alcohol use (Newlin and Thomson, 1990).

Sensitivity to an adverse effect of ethanol (e.g. ataxia and hypnosis) before AUD diagnosis is a pre-dependent response that may influence the amount of alcohol (ethanol) an individual consumes and may also predict future alcohol use patterns. A classic human study by Schuckit (1985) found that non-dependent men with a positive family history for alcoholism (FHP) were significantly less sensitive to ethanol as assessed by a measure of 'body sway' relative to those with a negative family history (FHN). The FHP/FHN distinction presumably reflects some degree of genetic difference. Furthermore, both this 'low level of response' (LLR) and FHP were found to be significant predictors of later alcohol problems (Schuckit, 1994), presumably because this insensitivity may allow individuals to consume larger quantities of alcohol before experiencing such adverse effects as ataxia.

The notion of LLR as a general AUD risk factor has been challenged (Newlin and Thomson, 1990), predominantly focusing on the timing of intoxication assessment following alcohol dosing. Studies that evaluated intoxication after full or nearly full alcohol absorption following administration (~30–60 min) have primarily found a variety of responses to alcohol to be attenuated in genetically at-risk individuals. Due to their timing, however, these assessments only evaluated intoxication on the descending limb of the time-BEC curve. Studies that evaluated intoxication at earlier time points following alcohol dosing, thus on the rising limb of the BEC curve, suggest that pre-dependent FHP individuals may actually exhibit *enhanced* sensitivity to a number of alcohol responses such as motor incoordination (Vogel-Sprott and Chipperfield, 1987), anxiolysis (Savoie et al., 1988), and subjective intoxication (Morzorati et al., 2002). Taken together, these findings suggest that those genetically predisposed for excessive alcohol consumption may exhibit heightened sensitivity to alcohol on the ascending limb and an attenuation of these responses on the descending limb (Newlin and Thomson, 1990).

The above observations are suggestive of an enhanced acute functional tolerance (AFT) capacity. AFT to adverse effects of alcohol may allow an individual to engage in more sustained drinking sessions. AFT, or within-session tolerance, is described as significantly reduced behavioral impairment at identical BECs on the descending limb of the time-BEC curve relative to the ascending limb (Kalant et al., 1971) (see Figure 1A for a graphical description). It is therefore important to evaluate responses to alcohol on both the ascending and descending limb to achieve the most complete assessment of potential individual differences in sensitivity to ethanol.

The existence of a genetic relationship between predisposition to sensitivity/tolerance and a propensity to consume alcohol in animal studies, however, is unclear. Rats and mice selectively bred for high alcohol consumption have been tested for hypnotic sensitivity and ataxia. Alcohol-preferring (P) and nonpreferring (NP) rats were not found to differ in initial sensitivity to a large, sedative-hypnotic dose of ethanol, although P rats developed significantly greater acute tolerance (Waller et al., 1983). On the other hand, it was recently found that selectively bred high alcohol-preferring (HAP) mice were significantly *more* sensitive to the ataxic effects of ethanol and also developed greater AFT to these effects relative to low alcohol-preferring (LAP) mice (Fritz et al., 2013). As measures of impairment, such as hypnosis and ataxia, are complex, different measures may evaluate only portions of these domains of intoxication (Crabbe et al., 2005). It is therefore important to evaluate a number of different intoxication measures to achieve a more complete picture of such alcohol responses and their relationships.

Furthermore, it is important to acknowledge that not all excessive alcohol consumption is the same; thus different sedative responses, regulated by different genes, may be more relevant to certain forms of problematic drinking. One relatively new genetic model of excessive alcohol consumption is the High Drinking-in-the-Dark (HDID) mouse. These mice have been selectively bred for high BECs following 20% ethanol consumption in the well-known binge-like ethanol consumption paradigm called 'Drinking-in-the-Dark' (DID) (Crabbe et al., 2009). The binge-like alcohol consumption of HDID mice, wherein significant alcohol consumption and intoxication is achieved in a short period of 2–4 hours, is markedly different from the continuous, sustained drinking of HAP mice which occurs throughout their active 12-hour circadian phase (Matson and Grahame, 2011).

Considering these genetic differences, it is of interest whether a genetic predisposition for *binge* alcohol consumption is associated with responses similar to those observed in HAP mice. HDID mice differ from their HS controls in some, but not all measures of acute ethanol sensitivity and tolerance. While all the tolerance assessments were made well after absorption and distribution of the ethanol injections were complete (Crabbe et al., 2012a), the sensitivity measures targeted both ascending and descending limbs of the BEC curve (Crabbe et al., 2012b). The objective of the current study was to characterize more completely sensitivity and AFT to the ataxic and hypnotic effects of ethanol in HDID mice of the first replicate of selection (HDID-1).

METHODS

Animals

Adult (PND 62–87) male and female HDID-1 (selection generation 25; estimated inbreeding coefficient = 0.298) and HS/Npt mice used in the current experiments were bred onsite at the Portland VA Medical Center. HS/Npt mice are a heterogeneous stock resulting from a systematic 8-way cross of inbred strains and are the founding population for the HDID selection and are thus used as a control for the HDID lines (details concerning the HS/Npt mice and the HDID selective-breeding procedures and heritability estimates can be found in Crabbe et al., in press). For this generation of HDID-1 (S25), average ethanol intake was measured at 6.2 ± 0.29 g/kg in a 4 hr DID test and BECs averaged 117 ± 9 mg/dl. HS/Npt

mice drink ~4.2 g/kg during the 4 hr DID test on average and reach BECs ~25 mg/dl. It must be noted, however, that the ethanol consumption of the HDID-1 and HS/Npt mice used in the current study is not known as these mice were required to be naïve at the time of testing.

Mice were shipped to the Psychology Department at Indiana University – Purdue University Indianapolis (IUPUI) for Experiments 1 and 2. Experiment 3 was conducted with animals on site at the Portland VA Medical Center. Mice shipped to IUPUI were allowed 2 weeks for acclimation before testing. At both institutions, mice were group-housed (2–5 per cage) and had *ad libitum* access to food and water. Holding rooms were maintained on a 12-h light/dark cycle (lights ON at 0900 at OHSU and 1400 at IUPUI) and temperature and humidity were held constant near 20° C and 50% respectively. All experiments were conducted during the light phase of the light/dark cycle and performed in naïve mice under a protocol approved by each location's Institutional Animal Care and Use Committee.

Ethanol Administration

One-hundred-ninety (Pharmco Inc., Brookfield, CT; Experiments 1 and 2) or two-hundred proof ethanol (Decon Laboratories Inc., King of Prussia, PA; Experiment 3) was diluted in sterile 0.9% physiological saline to a concentration of 15% v/v (Experiments 1 and 2) or 20% v/v (Experiment 3). Solutions were mixed fresh each test day. Ethanol was administered via intraperitoneal (i.p.) injection in weight-based doses of 1.75 g/kg, 2.0 g/kg (Experiments 1 and 2), or 2.75 g/kg (Experiment 3).

Static Dowel Task

The static dowel task was employed in Experiments 1 and 2 to evaluate initial sensitivity and AFT to ethanol's ataxic effects. The apparatus and procedures used were previously described in Fritz and colleagues (2013). Briefly, the task requires that the animal balance and maintain an upright position on a wooden dowel elevated (50 cm) and centered in a Plexiglas box (32×32×60 cm; 1 × w × h). Ethanol interferes with the ability to perform this task and 'loss of function' (LOF) is declared when the majority of the mouse's body swings below the imaginary horizontal plane that bisects the dowel (Fritz et al., 2013).

Modified Loss of Righting Reflex Task

The loss of righting reflex (LORR) task, where an animal is no longer able to right itself from a supine position, has long been used as a measure of the sedative-hypnotic effects of ethanol. However, the classic task requires the researcher to place a mouse on its back in a V-shaped trough for accurate determination of LORR, making it difficult to identify the precise moment at which the animal loses this reflexive ability. As we were interested in initial sensitivity and AFT, both of which require precise timing of the onset of LORR, we employed the apparatus and procedure developed by Ponomarev and Crabbe (2002). Essentially a modified restraint tube, the apparatus allows the researcher to continuously rotate the animal to evaluate whether or not LORR has been achieved.

Experiment 1: ‘Mellanby’ Assessment of Initial Sensitivity and AFT to Ethanol-Induced Ataxia Using the Static Dowel Task

This procedure is referred to as a ‘Mellanby’ assessment as it follows the original description of AFT by Mellanby in his work with canines (1919) where behavioral intoxication was observed to be markedly attenuated on the descending limb of the BEC curve relative to the ascending limb (hereafter referred to as ‘M-AFT’; see Figure 1A for a graphical description of the procedure). Mice were given 3, 1 minute training trials on the dowel before ethanol testing to ensure that they could perform the task. Mice were injected with a 1.75 g/kg dose of ethanol (15% v/v, i.p.) and immediately placed on the dowel. The time of LOF was recorded and a 20 µl blood sample was collected from the periorbital sinus. Consistent with the published literature reviewed above, initial sensitivity was defined as the BEC at which the target response (ataxia) was achieved. We wish to distinguish this from the definition of initial sensitivity set forth by Dixon and Massey (1968) wherein a dose of drug is titrated in order to determine the minimal effective dose producing the target response. Mice were retested at ~5 minute intervals after LOF and were deemed to have recovered if they were able to remain upright on the dowel for a full minute at which point another blood sample was collected. The time taken to recover is referred to as the duration of impairment (DOI). M-AFT was calculated by subtracting the BEC at LOF from the BEC at recovery. Care was taken to collect all blood samples within 8 seconds of each behavioral endpoint so as to minimally affect the true relationships between BEC and LOF or recovery.

Experiment 2: Two-Recovery Assessment of Initial Sensitivity and AFT to Ethanol-Induced Ataxia Using the Static Dowel Task

As the ‘Mellanby’ approach to AFT described above assesses tolerance that occurs very early in ethanol exposure, it was also of interest to determine whether a larger dose of ethanol over a longer period of time might produce line differences in more protracted acute tolerance. Therefore, we used the static dowel AFT procedure described by Erwin and Dietrich (1996). After LOF and recovery from an initial 1.75 g/kg injection, a second, larger dose of ethanol (2.0 g/kg) is administered immediately following the initial recovery on the dowel and animals are again retested until the recovery criterion is met a second time (see Figure 1B for a graphical description of the procedure). Blood samples were collected only after the first and second recovery and this index of AFT was defined as the difference between the BEC at recovery 2 and the BEC at recovery 1.

Experiment 3: ‘Mellanby’ Assessment of Initial Sensitivity and AFT to Ethanol-Induced Hypnosis Using the Modified LORR Procedure

Mice were weighed and injected with a 2.75 g/kg dose of ethanol (20% v/v, i.p.) and immediately placed into the appropriate restrainer based on body weight. The experimenter then turned the restrainer 90° every 2 seconds until the animal was unable to right itself for a total of 5 seconds (a criterion test had been performed on a separate group of animals to ensure that the animals did not lose their righting reflex simply from being turned 90° in the restrainer). Immediately following the LORR, the experimenter removed the mouse from the restrainer and obtained a blood sample from the periorbital sinus. Similar to Experiment 1, the BEC at LORR was interpreted as the index of initial sensitivity. The mouse was then

immediately returned to its restrainer and placed on its back. The restrainer was rotated 90° once the mouse began to move and if on the first turn following LORR the animal did not right itself within 2 seconds, the experimenter waited another 3–5 minutes to turn the restrainer again. If the animal righted itself within 2 seconds following the first 90° turn, the animal was again turned every 2 seconds for an additional 7 times before recovery was declared. Once a mouse recovered, it was immediately taken out of the restrainer and another blood sample was collected. M-AFT for this experiment was calculated by subtracting the BEC at LORR (LOF) from the BEC at recovery.

Blood Ethanol Concentration Determination

Blood samples from Experiments 1 and 2 were centrifuged and the plasma supernatant was siphoned off and transferred to 0.5 ml microcentrifuge tubes. Samples were stored at –80° C until determination of BEC in mg/dl by an Analox Alcohol Analyzer (Analox Instruments, Lunenburg, MA). Samples from Experiment 3 were analyzed via gas chromatography (specific details can be found in Rustay and Crabbe, 2004)

Statistical Analysis

All variables were analyzed via Analysis-of-Variance (ANOVA) with sex and genotype as factors or repeated measures ANOVA with measurement (LOF/LORR and/or recovery) as the additional factor. Analyses were collapsed on sex where it was not found to be a significant factor. BEC values at the relevant behavioral endpoints are graphically represented in each figure, but only the BEC at LOF/LORR (Experiments 1 and 3) or the BEC at recovery 1 (Experiment 2) were analyzed for group differences. This was done similarly to Fritz et al. (2013) to avoid unnecessary complication brought on by a repeated measures analysis as the relationship between both BEC values in each experiment is already described by M-AFT or AFT. The significance level for all analyses was set at $p < 0.05$ and Tukey-Kramer post-hoc statistics were run where applicable. Any main effects or possible interactions that are not reported were not found to be statistically significant. All analyses were conducted using Statistica 7 software (Statsoft).

RESULTS

Experiment 1: ‘Mellanby’ Assessment of Initial Sensitivity and AFT to Ethanol-Induced Ataxia Using the Static Dowel Task

Following the injection of ethanol, HDID-1 mice fell significantly later than HS/Npt mice ($F_{1,55} = 16.813, p < 0.001$; Figure 2A). The analysis of BEC at LOF confirmed that HDID-1 mice were indeed less initially sensitive to ethanol as their BEC values at LOF were significantly higher than those of HS/Npt mice ($F_{1,53} = 15.537, p < 0.001$; Figure 2B). A main effect of sex was also found for BEC at LOF ($F_{1,53} = 11.963, p < 0.01$), with LOF for males occurring at lower BECs than females (see Table 1 for group means split by sex). In addition to being less sensitive, HDID-1 mice recovered earlier than HS/Npt mice ($F_{1,53} = 29.212, p < 0.001$; Figure 2C). Furthermore, female mice recovered earlier than male mice ($F_{1,53} = 8.615, p < 0.01$; Table 1). Finally, there was no significant difference in M-AFT between genotypes ($p > 0.05$; Figure 2D), although a main effect of sex was found ($F_{1,53} = 9.565, p < 0.01$; Table 1). As mean M-AFT for female mice appeared to be near ‘0’, it did

not seem appropriate to statistically compare the sexes on this measure because both sexes would need to demonstrate M-AFT (difference in BECs significantly greater than 0) for accurate comparison. Follow-up t-tests comparing each of the group means to '0' determined that male mice developed a significant degree of M-AFT ($t_{27} = 3.925$, $p < 0.001$; two-tailed) whereas female mice did not ($p > 0.05$; Table 1). For the above main effects, none of the interactions with sex reach significance (all F 's < 2.279 , p 's > 0.137).

Experiment 2: Two-Recovery Assessment of Initial Sensitivity and AFT to Ethanol-Induced Ataxia Using the Static Dowel Task

Again, HDID-1 mice were found to fall significantly later than HS/Npt mice following the initial injection of ethanol ($F_{1,59} = 37.72$, $p < 0.001$; Figure 3A) and were also able to make their first recovery at higher BECs ($F_{1,57} = 23.629$, $p < 0.001$; Figure 3B). A main effect of sex was also found with female mice reaching recovery 1 at significantly higher BECs than male mice ($F_{1,57} = 19.403$, $p < 0.001$) (see Table 2 for group means split by sex). Main effects of genotype ($F_{1,57} = 40.975$, $p < 0.001$) and sex ($F_{1,57} = 18.544$, $p < 0.001$) were found for DOI with HDID-1 and female mice recovering earlier overall, respectively (Figure 3C). In addition, a significant genotype \times sex interaction was found ($F_{1,57} = 10.465$, $p < 0.01$) with post-hoc testing revealing that male HS/Npt mice took a particularly long time to recover ($p < 0.05$). Furthermore, a significant measurement \times genotype interaction was found ($F_{1,57} = 9.541$, $p < 0.01$) with the difference in DOI between HDID-1 and HS/Npt mice being greater at recovery 2 compared to recovery 1 ($p < 0.05$). Finally, there was no effect of genotype or sex on AFT ($p > 0.05$; Figure 3D).

Experiment 3: 'Mellanby' Assessment of Initial Sensitivity and AFT to Ethanol-Induced Hypnosis Using the Modified LORR Procedure

Following the injection of ethanol, there was no main effect of sex or genotype on LORR ($p > 0.05$; Figure 4A). However, a significant genotype \times sex interaction was found ($F_{1,82} = 6.097$, $p < 0.05$) with HS/Npt females losing their righting reflex earlier than HS/Npt males ($p < 0.05$; see Table 3 for group means split by sex). An analysis of BEC at LORR found that HDID-1 mice reached significantly higher BEC values at the point of LORR ($F_{1,84} = 4.515$, $p < 0.05$; Figure 4B) and were therefore less sensitive than HS/Npt mice. No significant effects were detected for DOI (p 's > 0.05 ; Figure 4C). A main effect of genotype was found for M-AFT ($F_{1,84} = 4.189$, $p < 0.05$). Similar to the analysis of Experiment 1, one group (HDID-1) appeared to have a M-AFT score near '0' thereby making a direct statistical comparison inappropriate. Follow-up t-tests comparing group means to '0' determined that HS/Npt mice developed a significant degree of M-AFT ($t_{44} = 2.842$, $p < 0.01$; two-tailed) whereas HDID-1 mice did not ($p > 0.05$; Figure 4D).

DISCUSSION

The findings of the current study highlight that HDID-1 mice are significantly less sensitive than HS/Npt mice to the ataxic and hypnotic effects of ethanol using these behavioral assessments. The evaluations of M-AFT capacity found only a minimal effect of genetic background with a small, but significant M-AFT capacity to ethanol-induced hypnosis in HS/Npt but not HDID-1 mice. No differences in AFT to the ataxic effects of ethanol were

detected at a higher combined dose or later time points as assessed by the two-recovery probe of the dowel test.

A recent investigation into the genetic relationship between selection for high or low alcohol preference in mice and ataxic sensitivity (using the same static dowel procedures as Experiments 1 and 2) found that selection for high alcohol preference was reliably genetically correlated with heightened sensitivity (Fritz et al., 2013). HDID-1 mice were also recently shown to be slightly more sensitive to acute ethanol than HS/Npt mice on a different measure of ataxia, foot slips on the parallel rod floor; however, HDID-2 mice did not differ from HS/Npt mice (Crabbe et al., 2012b). Given that murine ethanol intoxication is a complex construct (Crabbe et al., 2005), we chose to use the static dowel task to evaluate whether or not similar genes related to ataxic sensitivity in this task were captured by the HDID selection protocol. In addition, we chose to evaluate hypnotic sensitivity as it might also be considered an adverse consequence of the depressant effects of ethanol. HDID-1 mice were observed to be significantly less sensitive to both the ataxic and hypnotic effects of ethanol as evidenced by delayed LOF and shorter latency to recovery in Experiments 1 and 2 as well as correspondingly higher BEC values in all experiments. As such, these observations are likely due to genetic differences in pharmacodynamic sensitivity rather than ethanol pharmacokinetics. Indeed, a recent study found that HDID-1 and HS/Npt mice do not differ in their elimination rate of an acute dose (2.5 g/kg; i.p.) of ethanol (Crabbe et al., In press).

In order to better understand the comparison between these findings with HDID-1 versus the previous findings with HAP/LAP-2 and HAP/LAP-3, it is important to review the alcohol consumption phenotypes of these different lines of mice and explore whether there is genetic overlap in their drinking behavior. The three existing replicates of HAP mice consume large quantities of alcohol (~15–25 g/kg/day) and reach high BECs (HAP-1: ~225 mg/dl; HAP-2: ~100 mg/dl; HAP-3: ~80 mg/dl) in a continuous access paradigm offering a choice between 10% ethanol or water (Oberlin et al., 2011, Matson and Grahame, 2011). HDID mice of both existing replicates (HDID-1, HDID-2) consume ~7 g/kg of ethanol in a 4 hr DID test wherein the only fluid available is a 20% ethanol solution and reach BECs ~120–140 mg/dl (Crabbe et al., In press).

By evaluating the alcohol intake of HAP mice using DID procedures and HDID mice in a continuous access two-bottle choice paradigm, previous studies have explored the genetic overlap of these drinking phenotypes. Employing a slightly modified DID paradigm (daily 4 hr access to 20% v/v ethanol), HAP-1 mice (generation 46) were observed to consume high amounts of alcohol (~7.5 g/kg) whereas LAP-1 mice exhibited rather low intake (~2.5 g/kg) (Crabbe et al., 2011). It therefore appears that divergent genetic selection for high or low alcohol preference produced analogous drinking behavior in the limited access DID paradigm (although DID drinking behavior has not yet been evaluated in HAP/LAP-2 or HAP/LAP-3, the lines which were tested in Fritz et al. (2013)). When HDID-1 and HS/Npt mice have been tested for alcohol preference drinking in a continuous access 2-bottle choice paradigm with 10% alcohol, however, these lines consume fairly low doses (~4–6 g/kg/day) and there appears to be little effect of genetic background (Crabbe et al., 2011, Rosenwasser et al., 2013). Therefore, selective breeding for high BECs following binge-like drinking in

HDID-1 mice does not translate to excessive alcohol consumption or divergence from the HS/Npt progenitor stock in a continuous access paradigm, suggesting that these alcohol consumption behaviors are not equivalent and key genetic differences exist between the HDID and HAP lines with a preference for binge-like drinking evident in HDID-1 mice. As such, it is not particularly surprising that blunted initial sensitivity to these adverse effects of ethanol may be a particularly relevant factor for individuals predisposed to binge-drink, enabling them to consume large amounts of alcohol in a short period of time. On the other hand, predisposition for sustained excessive alcohol consumption may translate to an enhanced capacity to binge-drink, perhaps due to a rapid AFT capacity (Fritz et al., 2013) which could contribute to both forms of excessive alcohol consumption.

Interestingly, a recent study found HDID-1 mice to be less sensitive than HS/Npt mice to an ethanol-conditioned taste aversion (CTA), another task thought to reflect the negative hedonic effects of ethanol, produced by a moderate, 2.0 g/kg dose of ethanol. However, the genotypes did not differ in CTA produced by a high, 4.0 g/kg dose (Barkley-Levenson et al., epub ahead of print). A previous study exploring the genetic correlation between ethanol intake in DID and CTA in inbred mouse lines found a robust negative genetic correlation between these behaviors using the high, 4.0 g/kg dose of ethanol (Rhodes et al., 2007). However, a recent study by another group found no such genetic relationship in data obtained from wild type mice used to produce various genetic mutants with a lower (2.5 g/kg) dose of ethanol (Blednov et al., 2012). In conjunction with the CTA findings in HDID-1 and HS/Npt mice mentioned above, these observations suggest that the genetic relationship between these phenotypes is not yet clear and warrants further study.

The observation that the M-AFT and AFT capacity of HDID-1 was similar or even lower than HS/Npt mice may also not be all that surprising given their selection phenotype. The selection phenotype for HDID mice consists of 2 presentations of alcohol: one 2-hour DID access period on day 1 and a 4-hour access period on day 2 (Crabbe et al., 2009). Mice were selected this way because ethanol DID intake on day 2 strongly correlated with intake on subsequent days in studies with C57BL/6J mice, the inbred mouse strain with which the DID paradigm was developed (Rhodes et al., 2005). The previous observation of enhanced M-AFT capacity in ethanol-naïve HAP2 and HAP3 mice (Fritz et al., 2013) may be the result of a more protracted selection process as well as more extreme alcohol exposure wherein mice are phenotyped for alcohol preference in a continuous access paradigm over a 30-day period (Grahame et al., 1999). Therefore, the selection protocol for HDID may not have captured genes related to M-AFT or AFT due to a shorter duration of exposure or lesser total dose of alcohol during selection. It must also be noted, however, that the HAP/LAP and HDID mice were generated using different heterogeneous progenitor stocks (HS/Ibg for HAP/LAP and HS/Npt for HDID) and therefore, the different variety and frequency of alleles available for selection may have influenced the apparent differences observed between HAP-2/3 vs LAP-2/3 in the study by Fritz et al. (2013) and HDID-1 and HS/Npt in the current study.

When using a selective breeding approach in animal studies to evaluate genetic underpinnings of a complex phenotype of interest, it is important to determine whether or not differences observed between lines are, indeed, due to the genes captured by the

selection process and not the fixation of trait-irrelevant alleles (genetic drift). One way to address this issue is by testing additional lines of mice that have independently undergone the same selection process. If similar differences in a potential contributing phenotype (e.g. sensitivity or AFT) are maintained across replicate pairs of selected lines, this is considered strong evidence that common genes were captured by the selection process in each replicate and the non-selected trait is deemed a 'correlated response' to selection (Crabbe et al., 1990). Although a replicate line, HDID-2, exists, we did not test them here because they have been selectively bred for 7 fewer generations than HDID-1 and these lines may still be early enough in selection that an assessment of this correlated response is premature. Future planned work with HDID-2 mice will evaluate whether blunted ataxic and hypnotic sensitivity is indeed a strongly correlated response to HDID selection after several additional generations of selective breeding in these animals.

Taken together, these data suggest that HDID-1 mice model some aspects of the human LLR trait, insofar as binge-like drinking models a human AUD and the forms of ataxia and hypnosis we assessed model human body sway. Over the course of each static dowel assessment, HDID-1 mice reached LOF and recovery at significantly higher BECs than HS/Npt mice, indicating they were significantly less sensitive to this form of ethanol-induced ataxia on both the rising and falling limbs of the BEC curve. Our measure of ethanol-induced hypnosis (LORR) also detected lower initial sensitivity in HDID-1 mice on the ascending limb of the BEC curve. However, HDID-1 mice recovered their righting reflex at BECs similar to HS/Npt mice, suggesting that both genotypes may be equally sensitive to this intoxication measure on the descending limb of their BEC curves. As a previous study has demonstrated, HDID-1 mice do not exhibit a LLR to all acute effects of ethanol. HDID-1 mice were found to have a significantly greater acute locomotor stimulant response to ethanol than HS/Npt mice, although the lines did not differ in sensitivity to ethanol intoxication on a balance beam walking test (taken shortly after injection) or on the accelerating rotarod (assessed 30 min after injection) (Crabbe et al., 2012b). While HDID-1 mice exhibit a LLR to some adverse effects of ethanol, a recent evaluation of ethanol conditioned place preference suggests that their ethanol reward sensitivity is similar to HS/Npt mice (Barkley-Levenson et al., epub ahead of print). Therefore, aversive insensitivity may be a more relevant factor in the capacity of HDID mice to consume ethanol to intoxication in a binge fashion.

In conclusion, a genetic predisposition for high binge-like alcohol drinking was associated with reduced sensitivity to aspects of ataxia and hypnosis in HDID-1 mice, although they were relatively similar to HS/Npt mice in AFT capacity. This insensitivity may, in part, contribute to binge drinking susceptibility, allowing individuals to consume especially large quantities of alcohol in a short period of time before these adverse effects of intoxication are experienced. These findings differ from previous findings with the HAP and LAP selected mouse lines, suggesting that genetic predisposition for *binge*, versus other forms of excessive alcohol consumption, is associated with unique responses to ethanol-induced motor incoordination; responses which may be regulated by genes specifically contributing to binge alcohol drinking risk.

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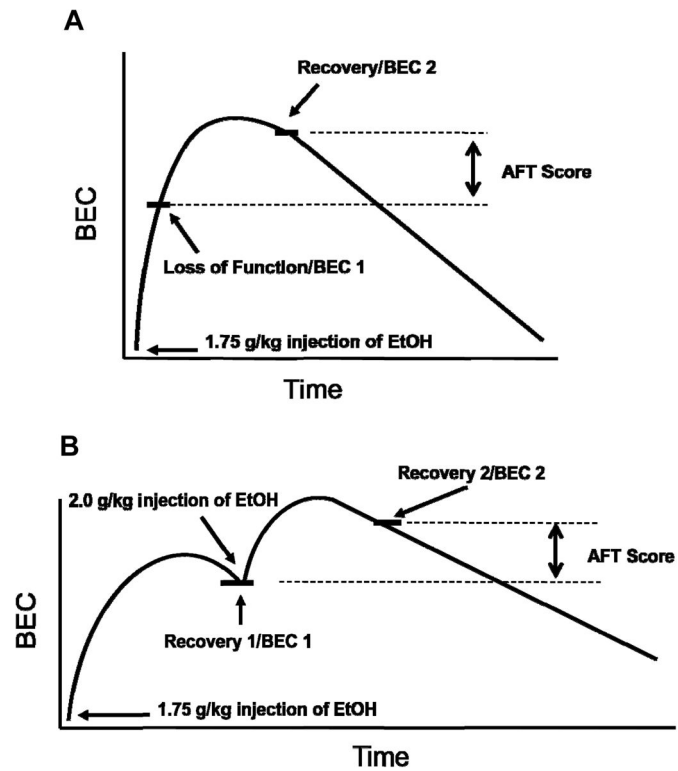


Figure 1.

Graphical representation of procedures used for the static dowel assessment of initial sensitivity and AFT to the ataxic effects of ethanol. A) The 'Mellanby' approach to AFT used in Experiments 1 and 3. B) The 'two-recovery' approach of Erwin and Dietrich used in Experiment 2.

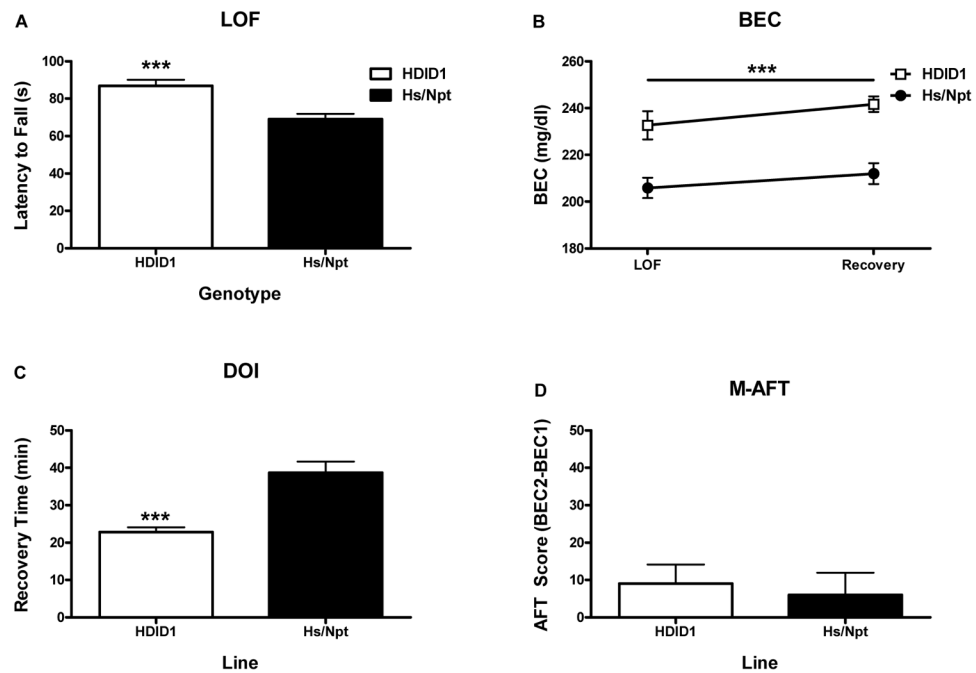


Figure 2.

(Experiment 1): Static dowel assessment of sensitivity and AFT to the ataxic effects of ethanol following a single 1.75 g/kg injection of 15% EtOH in HDID1 and HS/Npt mice ($n = 13-15$ per sex/line). A) Time taken for mice to lose balance on the dowel following the injection of ethanol. B) Blood ethanol concentrations (BECs) at LOF and recovery. C) Duration of impairment (DOI): time taken following the ethanol injection for mice to reach recovery criterion. D) ‘Mellanby’ assessment of acute functional tolerance (M-AFT), calculated by subtracting the BEC measurement at LOF from the BEC measurement at recovery. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

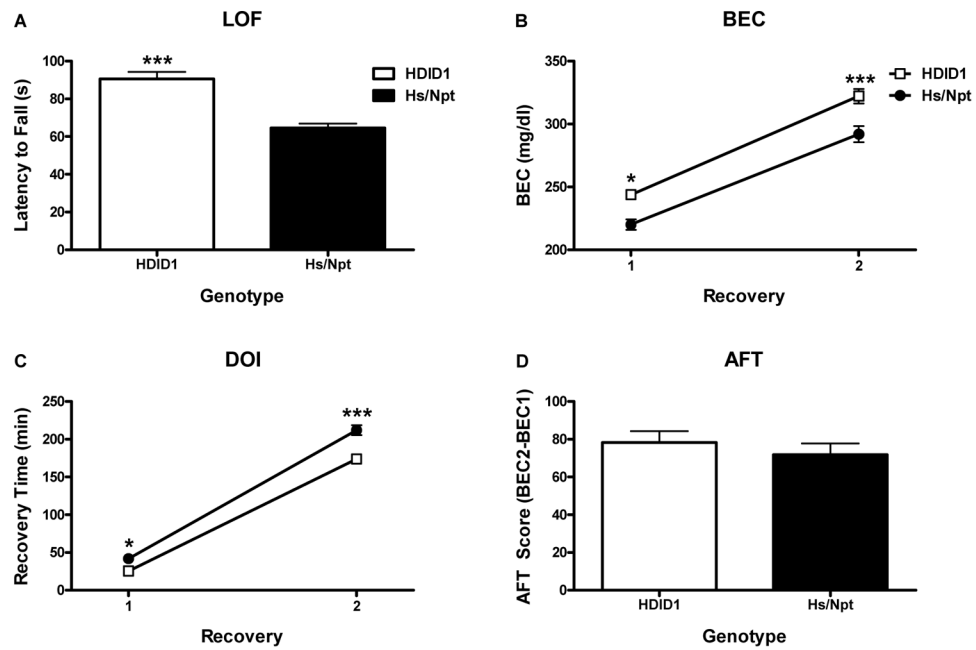


Figure 3.

(Experiment 2): Static dowel assessment of sensitivity and AFT to the ataxic effects of ethanol using the 2-recovery approach in HDID1 and HS/Npt mice ($n = 15-16$ per sex/line). A) Time taken for mice to lose balance on the dowel following the initial injection of ethanol. B) Blood ethanol concentrations (BECs) at recovery 1 and recovery 2. C) DOI: time taken following the initial ethanol injection for mice to reach recovery criterion. D) Acute functional tolerance (AFT), calculated by subtracting the BEC measurement at recovery 1 from the BEC at recovery 2. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

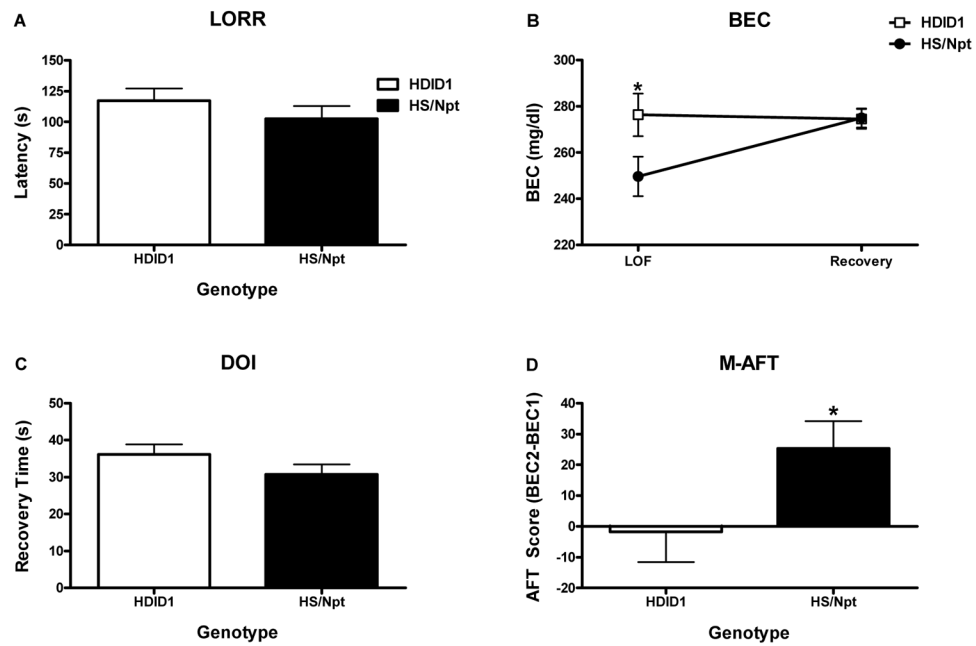


Figure 4.

(Experiment 3): Assessment of sensitivity and AFT to the hypnotic effects of ethanol following a 2.75 g/kg injection of EtOH in HDID1 and HS/Npt mice ($n = 15-16$ per sex/line). A) Time taken for mice to lose their righting reflex following the injection of ethanol. B) Blood ethanol concentrations (BECs) at LORR and recovery. C) DOI: time taken following the ethanol injection for mice to reach recovery criterion. D) ‘Mellanby’ assessment of acute functional tolerance (M-AFT), calculated by subtracting the BEC measurement at LORR from the BEC measurement at recovery $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table 1

“Mellanby” assessment of static dowel acute functional tolerance.

Line	Sex	n	LOF (sec)	DOI (min)	BEC1 (mg/dl)	BEC2 (mg/dl)	M-AFT (BEC2-BEC1)
HDID-1	M	15	84.7 ± 6.3 ***	28.3 ± 1.4## ***	215.7 ± 9.1## ***	238.7 ± 4.8 ***	23.1 ± 6.6##
	F	15	88.9 ± 2.6	17.3 ± 1.0	249.7 ± 5.3	244.7 ± 4.8	-5.0 ± 6.0
HS/Npt	M	13	67.5 ± 3.1	42.0 ± 5.2##	199.0 ± 5.7##	213.8 ± 6.8	14.8 ± 7.4##
	F	14	70.6 ± 4.6	35.6 ± 3.1	212.4 ± 6.2	210.3 ± 6.1	-2.1 ± 8.9

$p < 0.001$ versus HS/Npt (main effect of genotype),

##

$p < 0.01$ versus females (main effect of sex).

Table 2

Two-recovery assessment of static dowel acute functional tolerance.

Line	Sex	n	LOF (sec)	DOI-R1 (min)	DOI-R2 (min)	BEC1 (mg/dl)	BEC2 (mg/dl)	AFT (BEC2-BEC1)
HDID-1	M	15	90.3 ± 5.0 ***	28.5 ± 2.6###	175.2 ± 5.6 ***	233.5 ± 5.2###	315.5 ± 7.7 ***	81.8 ± 8.1
	F	15	90.9 ± 5.3	22.4 ± 1.5	172.3 ± 7.1	254.1 ± 3.5	328.8 ± 8.7	74.7 ± 9.2
HS/Npt	M	16	61.6 ± 3.5	52.7 ± 3.8###	232.0 ± 7.3\$\$\$	209.5 ± 5.0###	290.1 ± 9.2	80.6 ± 8.4
	F	15	67.8 ± 2.9	30.7 ± 2.1	190.7 ± 8.6	231.5 ± 5.3	293.9 ± 9.1	62.4 ± 8.2

 $p < 0.001$ versus HS/Npt (main effect of genotype);

###

 $p < 0.001$ versus females (main effect of sex);

\$\$\$

 $p < 0.001$ vs. all other line/sex combinations.

Table 3

“Mellanby” assessment of LORR acute functional tolerance.

Line	Sex	n	LOF (sec)	DOI (min)	BEC1 (mg/dl)	BEC2 (mg/dl)	M-AFT (BEC2-BEC1)
HDID-1	M	18	109.4 ± 11.9	35.0 ± 3.9	266.1 ± 10.0 *	271.0 ± 6.0	4.9 ± 9.7 *
	F	23	123.6 ± 15.0	36.9 ± 4.0	284.2 ± 14.3	277.3 ± 6.0	-6.9 ± 15.9
HS/Npt	M	21	131.6 ± 18.4@	27.7 ± 4.0	260.5 ± 13.7	274.4 ± 6.8	13.9 ± 14.0
	F	24	77.0 ± 8.3	33.3 ± 3.8	240.2 ± 10.6	275.5 ± 4.9	35.3 ± 11.2

* $p < 0.05$ versus HS/Npt (main effect of genotype);

@ $p < 0.05$ versus HS/Npt females.